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TOXICITY STUDIES OF TRIAMINO GUANIDINE NITRATE

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TECHNICAL REVIEW AND APPROVAL

AMRL-TR-77-16

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



ANTHONY A. THOMAS, MD

Director
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The acute toxicity of triaminoguanidine nitrate (TAGN) has been studied. The LD50/14 of TAGN in mice was 3.65 g/kg with 95% confidence limits of 3.54 - 3.76 g/kg. At IV doses of 50-200 mg/kg given to anesthetized dogs, TAGN caused a slightly decreased heart rate and a transient fall in arterial blood pressure followed by a slight increase. In these animals, TAGN did not alter the function of the peripheral autonomic nervous system or the neuromuscular junction. Based on the limited results of this study and the classification system of Hodge and Sterner (8), TAGN belongs in the category of slightly toxic compounds.		

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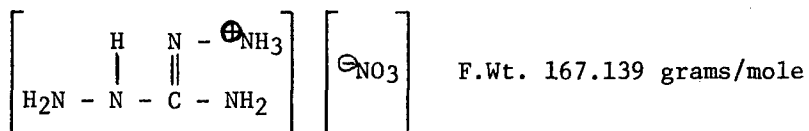
PREFACE

The research presented in this report was performed by members of the Toxicology Branch, Toxic Hazards Division, Aerospace Medical Research Laboratory from August 1976 through October 1976. This research was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials;" Tasks 630201, "Toxicology of Propellants and Materials," and 630202, "Procedures for Diagnosis and Treatment of Air Force Exposure Cases;" Work Units 63020104 and 63020213.

The authors acknowledge the assistance of Major Norman R. Schneider and Sgt William P. J. Klemm during this study.

INTRODUCTION

Triaminoguanidine nitrate (TAGN) is being tested by the Air Force Armament Laboratory for use as a component of propellants. The chemical structure and formula weight of TAGN are indicated below:



No information about the toxicity of TAGN has been found in the literature. However, several recent reports have shown that guanidine and derivatives of guanidine produce effects on the autonomic nervous system (Turnbull et al., 1976; Misu et al., 1976; Shah et al., 1976; and Baum and Shropshire, 1976). Guanidine derivatives also interact with histamine receptors (Rezende et al., 1976) and the neuromuscular junction (Volle and Branesteanu, 1976).

The purpose of this study was to assess the toxic hazard of acute exposure to TAGN. To do this, the LD_{50/14} of TAGN was determined as an index of its toxicity. Also, because of the reported effects of other guanidine derivatives, an experiment was done to screen TAGN for acute effects on the function of the autonomic nervous system.

METHODS

LD_{50/14} Determination. Male CD-1 mice were given intraperitoneal injections of TAGN dissolved in physiological saline (maximum concentration of 80 mg/ml). The TAGN concentration was adjusted to administer to groups of 20 mice, doses of 3.0, 3.2, 3.4, 3.6, and 3.8 g/kg using an injection volume of 0.01 ml/g body weight. Nine mice were given 4.0 g/kg. Small groups of mice were given greater doses in preliminary rangefinding studies. An equivalent volume of saline was administered to three mice serving as controls. After injection, the mice were placed in separate cages and observed for eight hours. The mice were then placed in group cages and held for two weeks. The LD_{50/14} was calculated using the log dose-probit analysis method of Litchfield and Wilcoxon (1949).

Screen for Effects on Autonomic Nervous System. Five dogs were anesthetized with Dial®-urethane (contains allobarbitol 100 mg/ml, urethane 400 mg/ml, mono-ethylurea 400 mg/ml in water) given intravenously at an approximate dose of 0.6 ml/kg to reach a surgical plane. The trachea was intubated and the animal respired with room air. Arterial blood pO₂, pCO₂, and pH were monitored to assure adequate ventilation. The dogs were instrumented to measure and record arterial blood pressure, lead II EKG, heart rate and contraction of the nictitating membrane. A cervical portion of the right vagus nerve was isolated, cut, and each cut end prepared for electrical stimulation. In one dog the left vagus was left intact so that responses due to cardiovascular reflex mechanisms could be observed, but in all others it was cut. The femoral vein in one leg was catheterized for intravenous injections. Two of the five dogs were also prepared to study the effects of TAGN on neuromuscular junction transmission. The gastrocnemius tendon in one leg of these dogs was dissected free, cut and attached to a strain gauge transducer. The sciatic nerve was isolated proximal to the stifle and stimulated with supramaximal voltage to cause contraction of the gastrocnemius muscle.

After the preparation had stabilized, a control response to the following was obtained: IV injection of tyramine (20 µg/kg), norepinephrine (1 µg/kg), epinephrine (1 µg/kg), and acetylcholine (50 µg/kg), and electrical stimulation of the central and peripheral cut ends of the right vagus nerve. Sufficient time was allowed between each injection and stimulation for all recorded activity to return to baseline levels. Various doses of TAGN were then administered by IV injection and the responses to injection of the above compounds and stimulations were recorded.

RESULTS

LD50/14 Determination. The mice given TAGN at 3.0 to 4.0 g/kg were lethargic and appeared ill with labored breathing. No other overt signs of toxicity occurred during the time they were observed. Death occurred between 6 and 24 hours after injection. The animals given higher doses (4.8 and 5.6 g/kg) during rangefinding studies died within 30 to 60 minutes. Toxic symptoms preceding death included labored breathing, shivering and then tonic and clonic convulsions. The control group of mice appeared normal at all times.

The regression function for probit lethality versus log dose is probit lethality = 22.059 (log dose) - 73.568. Using this equation, the calculated LD50 is 3.65 g/kg with 95% confidence limits of 3.54 - 3.76 g/kg.

Physiological Effects of TAGN. At 1 and 10 mg/kg, TAGN produced no measurable change in any of the physiological parameters recorded. TAGN at 50 mg/kg and higher caused a decreased heart rate and a transient fall in arterial blood pressure followed by a slight increase over baseline. These changes were very small with 50 mg/kg. A dose of 100 mg/kg produced a 17% increase of the time between successive heart beats (our measure of heart rate) and approximately a 10% increase in both systolic and diastolic blood pressure. The duration of these effects was 20 to 25 minutes and response to 200 mg/kg was no greater than the response to 100 mg/kg.

TAGN did not affect any of the measures of autonomic function. It did not cause a statistically significant change in the response to electrical stimulation of the sciatic nerve or the cut ends of the right vagus nerve. Nor did TAGN alter the response to intravenous injection of tyramine, norepinephrine, epinephrine and acetylcholine. The above statements are true also for the dog in which the left vagus nerve was intact.

DISCUSSION

Based on the limited results of this study, we conclude that triamino-guanidine nitrate is a relatively non-toxic chemical. However, one must use caution in extrapolating animal data to man because man may not react as other species do. If equal sensitivity is assumed, a 70 kg man would have to be exposed to an acute dose of 250 grams (over half a pound) of TAGN to produce a similar response. Based on the classification system of Hodge and Sterner (1953) TAGN is in the category of slightly toxic compounds.

TAGN did have a small effect on heart rate and arterial blood pressure. However, the dose required to produce these effects was also relatively high. Man would require seven grams of TAGN if he were as sensitive to these effects as the dog.

In contrast to the reported effects of other guanidine derivatives, TAGN did not effect the responses to autonomic agonists. Therefore, TAGN does not significantly alter autonomic nervous system function.

The primary purpose of this study has been fulfilled. Nevertheless, one should realize that TAGN may produce other acute or chronic effects for which we have not tested. Such effects as skin and eye irritation, hypersensitivity reactions, carcinogenicity, mutagenicity and teratogenicity could confound the total assessment of hazard. If TAGN is to be used in relatively large quantities, then these other parameters should be evaluated.

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